CONCISE COMMUNICATION

Trends in Human Immunodeficiency Virus Type 1 (HIV-1) Load among HIV-1–Infected Children with Hemophilia

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In human immunodeficiency virus type 1 (HIV-1)–infected persons, virus load (serum/plasma level of HIV) predicts outcome. Virus load trends have been characterized in adults and infants but not in children. Virus load trends in 22 male children with hemophilia who acquired HIV-1 postnatally (age 0.7–5.2 years at seroconversion) were studied. The mean HIV-1 load 2 years after seroconversion was 4.40 $\log_{10}$ copies/mL, and the mean change over time (slope) was 0.03 $\log_{10}$ copies/(mL · year). Significant among-children variation was apparent: a random effects model predicted that 95% of children had early virus loads 3.75–5.04 $\log_{10}$ copies/mL and slopes −0.07 to 0.12 $\log_{10}$ copies/(mL · year). Higher early virus loads and higher slopes were each associated with increased mortality ($P = .006$ and $P = .03$, respectively). In conclusion, those subjects had virus load trends similar to those in adults. Early virus loads were lower than those in vertically infected infants, which suggests that factors changing soon after birth affect viral replication.

Human immunodeficiency virus type 1 (HIV-1) replication generally persists throughout infection. The level of circulating HIV-1 measured in plasma or serum (virus load) reflects the degree of HIV-1 replication in lymphoid tissue and predicts the risk of AIDS and death [1–5]. Combination antiretroviral treatment can suppress replication for prolonged periods, dramatically improving outcome.

Adults and vertically infected infants differ in their virus load patterns over time. Among untreated HIV-1–infected adults, early virus loads (measured 12–36 months after seroconversion) are typically 1000–10,000 copies/mL (3.00–4.00 $\log_{10}$ copies/mL) [2]. Virus loads (when considered as log-transformed values) tend to increase slowly over time, although adults vary in their rates of change (slopes) [6, 7]. In contrast with adults, vertically infected infants have higher early virus loads; unlike adults, their virus loads gradually decline from these early values [4, 5, 8].

Reasons for differences between infants and adults are unknown. Children who become HIV-1 infected outside the perinatal period, a group with an intermediate age at infection, can provide information on age-related determinants of viral replication. However, there are no published reports on virus load trends in this group. Therefore, we studied young children with hemophilia, who became HIV-1 infected through infusions of HIV-1–contaminated clotting factors. Much of their clinical course occurred before effective combination antiretroviral therapy became available, so we could observe patterns in virus load that were largely unaffected by treatment.

Subjects and Methods

Subjects and clinical outcomes. The Multicenter Hemophilia Cohort Study (MHCS) is a prospective cohort study of hemophilia subjects enrolled at 16 treatment sites in the United States and Europe, beginning in 1984 [9]. Enrolled subjects are seen every 6–12 months. HIV-1 seroconversion dates previously were estimated by using individuals' HIV-1 test results and data on use of clotting factors [10]. For this study, we included MHCS subjects who were HIV-1 infected by clotting factor products at age <6 years. Included subjects had available for virus load testing ≥2 stored serum or plasma samples obtained ≥1 year apart. In addition, the first available sample for included subjects had been obtained within 2.5 years after seroconversion.

Laboratory measurements. Plasma or serum samples were separated from whole blood and were frozen on the same day (2–8 h after sampling) or after overnight shipment. Specimens were stored at −70°C until tested. We measured virus loads (Amplicor

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Informed consent was obtained from the parents or guardians of subjects in this study. This study complied with human experimentation guidelines of the US Department of Health and Human Services and was reviewed by Institutional Review Boards at the National Cancer Institute and participating institutions.

[a] Multicenter Hemophilia Cohort Study participants and institutions are listed after the text.

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HIV Monitor assay; Roche Molecular Systems) in the first available sample after HIV-1 seroconversion and subsequent samples ~1 year apart in time. Heparin, a potential inhibitor, was removed from plasma samples by use of silica extraction. A value of 100 copies/mL was assigned to readings below the assay's threshold of 200 copies/mL, and results obtained from serum were multiplied by 1.32 to make them comparable with plasma values [7].

For each subject, we analyzed all available measurements of the percentage of lymphocytes that were CD4 positive (CD4%). We used CD4% values instead of absolute CD4 lymphocyte counts, because the total lymphocyte count decreases substantially during normal childhood. Measurements were performed on monoclonally stained lymphocytes, using flow cytometry.

Statistical methods. For descriptive purposes, we fitted time trends to each subject's $\log_{10}$-transformed virus load, using linear regression ("observed" trends). This approach is convenient, but it inappropriately assumes that, apart from the time trend, successive observations for any individual are uncorrelated. We fitted a random-effects model to study virus load trends more formally [11]. Under this model, the $\log_{10}$ virus load for subject i at year t_{ij} (denoted Y_{ij}) was modeled as $Y_{ij} = \beta_{0i} + \beta_{1i}(t_{ij} - 2) + \varepsilon_{ij}$, where β_{0i} was the ith subject's early $\log_{10}$ virus load and β_{1i} was the ith subject's virus load slope. This model was centered so that β_{0i} values were the expected $\log_{10}$ virus loads at 2 years after seroconversion. The early virus loads (β_{0i} values) and slopes (β_{1i} values) could vary among individuals and were each considered to be normally distributed. For each individual i, we modeled the serial correlation among the errors ϵ_{ij} with a negative exponential function [11].

Using the random-effects model, we estimated the population means for early virus load and slope, which provided an estimate of the average trend. We also assessed whether individuals differed in their time trends by testing whether the variance among individuals in early virus loads or slopes was significantly greater than zero. In addition, we evaluated models with terms for age at HIV-1 seroconversion and antiretroviral therapy at time t_{ij} (any vs. none).

The random-effects model gave estimates for each child's early virus load and slope. These estimates for each individual (the separate estimates for β_{0i} and β_{1i}) were each weighted averages of that individual's observed values and the population means, so that the estimates were pulled toward the means.

We performed similar modeling for CD4% measurements. To examine the relationship between early viral replication and subsequent loss of CD4 lymphocytes, we calculated the Pearson correlation coefficient between random-effects estimates of early virus loads and CD4% slopes. In other analyses, we used the Wilcoxon rank sum and log-rank tests to compare continuous outcomes and incidence rates, respectively, between groups. Results were considered to be statistically significant ($P \leq .05$) or borderline significant ($P = .06$–$.10$).

Results

Study subjects. Eighty-nine children had HIV-1 seroconverted at age <6 years. Of these 89 children, 22 (25%) had sufficient serial serum or plasma samples for inclusion in the present study (most excluded subjects lacked samples within 2.5 years of seroconversion). For the 22 children who were included (all males), the median age at seroconversion was 3.4 years (range, 0.7–5.2 years). Included subjects seroconverted between 1981 and 1985. During 270 person-years after seroconversion, 6 children developed AIDS-associated opportunistic illnesses, and 5 died. Compared with children in the study, excluded children had similar ages at seroconversion ($P = .95$), incidence of opportunistic illness ($P = .74$), and mortality ($P = .38$).

Virus load measurements. Included children had 150 virus load measurements (median, 6 measurements/subject; range, 4–10 measurements/subject; median, 1.4 years between measurements), with initial measurements at a median of 2.0 years after seroconversion (interquartile range, 1.0–2.2). Figure 1 displays virus load measurements for 10 representative subjects and "observed" time trends fitted with linear regression. These trends fitted most subjects' measurements well, although substantial variation was apparent for some subjects (e.g., subjects E and G).

Under a random-effects model, the mean early virus load was 4.40 $\log_{10}$ copies/mL (95% confidence interval [CI], 4.14–4.66) and the mean virus load slope was 0.03 $\log_{10}$ copies/(mL · year; 95% CI, −0.02 to 0.07). The population mean trend derived from these values also is shown in figure 1. Individual observed trends varied markedly around this mean trend (figure 1). Indeed, variances among subjects in early virus loads and slopes were each significantly greater than zero when considered separately ($P = .05$ for each), and together they were borderline significant ($P = .07$), which implies the presence of true differences among subjects. The model estimated that 95% of individuals had early virus loads between 3.75 and 5.04 $\log_{10}$ copies/mL and virus load slopes between −0.07 and 0.12 $\log_{10}$ copies/(mL · year).

The random-effects model also provided separate estimates for each individual's virus load trend (figure 1). Observed and random-effects trends agreed for most subjects. However, for a few subjects the observed early virus loads or slopes appeared to be extreme (subjects B and J), and their random-effects trends were pulled toward the population mean.

Age at seroconversion was not significantly related to early virus load or slope ($P = .37$ and $P = .18$, respectively). Subjects were receiving antiretroviral therapy at 30 time points (20%), most of which was monotherapy (23 time points; 77%). Virus load was not related to antiretroviral therapy (mean virus load, 4.70 $\log_{10}$ copies/mL with therapy vs. 4.46 $\log_{10}$ copies/mL without therapy; $P = .58$).

As shown in figure 2*A* and 2*B*, high early virus load and high virus load slope were each significantly associated with increased mortality.

CD4% measurements. There were 334 CD4% measurements (median 17 measurements/subject; range, 2–32 measurements/subject; median, 0.5 years between measurements). Under a random-effects model, the mean early CD4% (2 years after seroconversion) was 31% (95% CI, 28%–34%), and the mean CD4% slope was −1.7% per year (95% CI, −2.3% to −1.2%). Under this model, all children had the same early CD4% (namely, 31%); however, uncertainty in this prediction

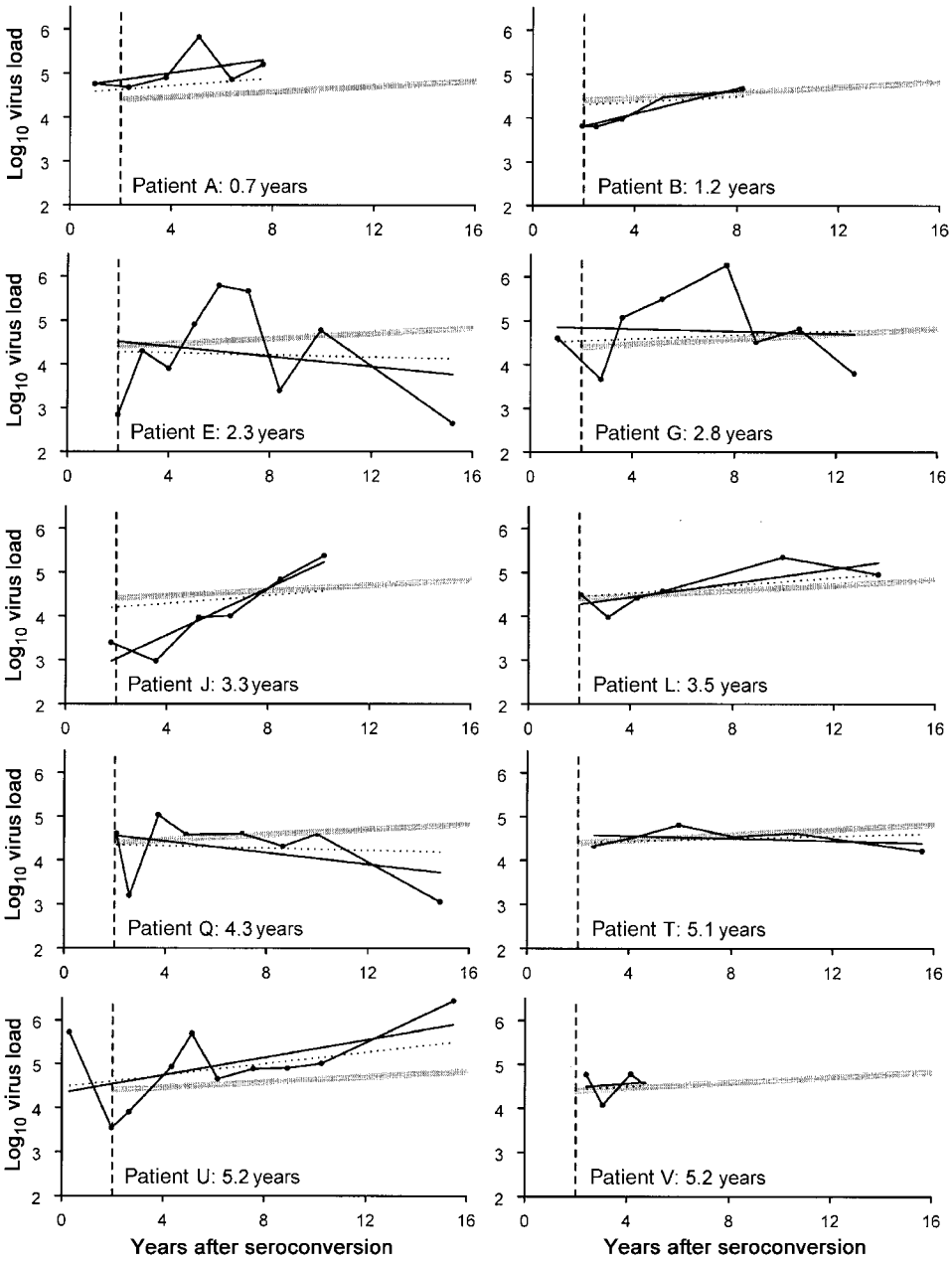

Figure 1. Virus load measurements for 10 representative human immunodeficiency type 1 (HIV-1)–infected children. For each subject, serial virus load observations (log_{10} copies/mL) are indicated by points connected by solid black lines. For each subject, the single solid black line drawn across all observations indicates the time trend derived by using linear regression (referred to in the text as the "observed" time trend). The dotted line indicates the time trend fitted by using the random-effects model, and the gray line indicates the population average trend (same in each panel). The vertical dashed line, at 2 years after seroconversion, marks the time for which early virus loads were estimated. Subjects are ordered by their age at HIV-1 seroconversion, which is noted at the bottom left of each panel.

allowed for differences among children: with 95% confidence, the model predicted that 95% of children had early CD4% values between 17% and 47%. Variation in CD4% slopes was significant ($P = .003$), and 95% of individuals were predicted to have slopes between −3.1% and −0.4% per year.

There was a nonsignificant, negative correlation between early virus loads and CD4% slopes ($R = -.18$; $P = .40$).

Discussion

In those children who acquired HIV-1 infection through clotting factor infusions, HIV-1 load trends resembled trends previously reported for adults [2, 6, 12]. We found that the mean virus load was 4.40 log_{10} copies/mL 2 years after seroconversion. In comparison, for MHCS subjects who HIV-1–serocon-

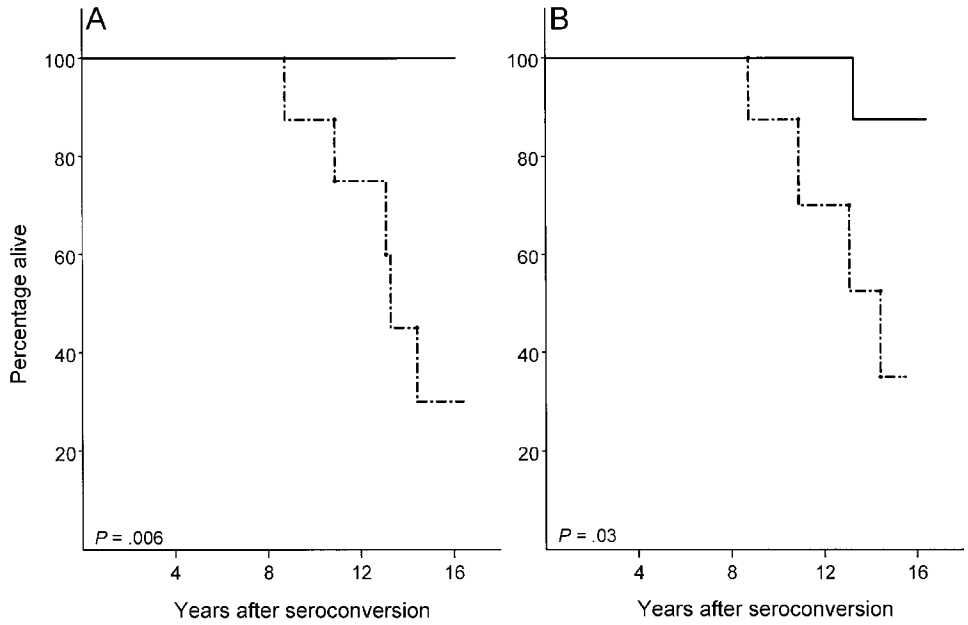

Figure 2. Survival of human immunodeficiency virus type 1 (HIV-1)–infected children as a function of random-effects estimates for early virus load *(A)* or virus load slope *(B)*. In each panel, Kaplan-Meier plots are shown, with subjects divided at the median value (4.40 $\log_{10}$ copies/mL for early virus load; 0.03 $\log_{10}$ copies/[mL·year] for virus load slope). In each panel, the solid line corresponds to children with values below the median, and the dashed line corresponds to children with values above the median. The difference in survival between the 2 groups is significant in both analyses (*P* values shown).

verted at 35–70 years old, the median virus load 12–36 months after seroconversion was 4.08 $\log_{10}$ copies/mL [2]. After this early period, virus loads in our subjects tended to increase slowly (mean slope, 0.03 $\log_{10}$ copies/[mL·year]). This increase was similar to, but slightly more gradual than, increases reported for adults (0.03–0.09 $\log_{10}$ copies/[mL·year]) [6, 7, 12].

In contrast with these children and with adults, perinatally infected children have higher virus loads early in infection (typically averaging 5.00–5.20 $\log_{10}$ copies/mL at 24–36 months of age), and their virus loads tend to decline subsequently [4, 5, 8]. These differences suggest that critical changes occur in the first year of life that can affect early viral replication. Cell-mediated immunity may be important in this regard [13]. This system is immature at birth but, by age 1–5 years, may be able to control early viremia. Also, in fetuses and young infants, HIV-1 readily infects the thymus, which might augment plasma levels of HIV-1 [14]. Thymic involution begins after the neonatal period and could lead to lower virus loads with later infection. Similarly, absolute levels of CD4 lymphocytes are high in infancy, which could facilitate viral replication. We could not distinguish among these possibilities in our study.

High early virus load and steeply positive slopes were associated with elevated mortality. We also found an inverse, though nonsignificant, relationship between early virus load and CD4% slope. HIV-1–mediated destruction of CD4 lymphocytes may partly account for the deleterious effects of high HIV-1 load [1]. Nonetheless, HIV-1 replication can increase risk for disease progression independently of its effects on CD4 lymphocyte levels [3, 15].

We noted significant variation among children in virus load trends. For example, some children had positive slopes, and others had negative slopes. Possible reasons for this variation include qualitative or quantitative differences in the virus inoculated through contaminated factor infusions or genetic differences among children. Because individual trends truly differ among children, the population average trend is only partly informative.

For subjects who had few data or extreme observed trends, the random effects estimates were pulled toward the population average. In this way, our random effects model appeared to correct potentially unreliable observations. Notably, random effects estimates of early virus load and slope strongly predicted mortality, which suggests that the model was reasonable. These relationships were less apparent when observed trends were used instead (data not shown).

Our study is unique in its description of individuals who acquired HIV-1 infection in early childhood. In light of the difficulty in identifying informative subjects, its main limitation is the small number of children studied. Included children may have differed from excluded children, although we found no differences in age at seroconversion, incidence of opportunistic illnesses, or mortality. Our study included only male children, so our results conceivably might not apply to females. Also, because we had few virus load measurements within the first year after seroconversion, we were unable to characterize virus load patterns during

primary infection. Finally, we had few data on combination antiretroviral therapy, because most follow-up occurred before 1996. Nonetheless, we did not see an effect of less effective therapy (mostly monotherapy) on virus loads, and our study design allowed us to observe biologically relevant trends that would have been obscured by more effective therapy.

In conclusion, HIV-1 load patterns in children with hemophilia more closely resemble patterns in adults than those in vertically infected infants. Among these children, as in other groups, HIV-1 loads provide prognostic information. Differences in trends among children justify continued attempts to identify host and viral determinants of disease progression.

Institutions and Investigators in the Multicenter Hemophilia Cohort Study

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References

1. Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4$^+$ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med **1997**;126:946–54.
2. O'Brien TR, Blattner WA, Waters D, et al. Serum HIV-1 RNA levels and time to development of AIDS in the Multicenter Hemophilia Cohort Study. JAMA **1996**;276:105–10.
3. Engels EA, Rosenberg PS, O'Brien TR, Goedert JJ. Plasma HIV viral load in patients with hemophilia and late-stage HIV disease: a measure of current immune suppression. Ann Intern Med **1999**;131:256–64.
4. Palumbo PE, Raskino C, Fiscus S, et al. Predictive value of quantitative plasma HIV RNA and CD4$^+$ lymphocyte count in HIV-infected infants and children. JAMA **1998**;279:756–61.
5. Mofenson LM, Korelitz J, Meyer WA, et al. The relationship between serum human immunodeficiency virus type 1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1–infected children. J Infect Dis **1997**;175:1029–38.
6. Hubert J, Burgard M, Dussaix E, et al. Natural history of serum HIV-1 RNA levels in 330 patients with a known date of infection. AIDS **2000**;14:123–31.
7. O'Brien TR, Rosenberg PS, Yellin F, Goedert JJ. Longitudinal HIV-1 RNA levels in a cohort of homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol **1998**;18:155–61.
8. Biggar RJ, Janes M, Pilon R, et al. Virus levels in untreated African infants infected with human immunodeficiency virus type 1. J Infect Dis **1999**;180:1838–43.
9. Goedert JJ, Kessler CM, Aledort LM, et al. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. N Engl J Med **1989**;321:1141–8.
10. Kroner BL, Rosenberg PS, Aledort LM, Alvord WG, Goedert JJ. HIV-1 infection incidence among persons with hemophilia in the United States and Western Europe, 1978–1990. J Acquir Immune Defic Syndr **1994**;7:279–86.
11. Diggle PJ, Liang K, Zeger SL. Analysis of longitudinal data. Oxford: Oxford University Press, **1994**:33–189.
12. Lyles CM, Dorrucci M, Vlahov D, et al. Longitudinal human immunodeficiency virus type 1 load in the Italian Seroconversion Study: correlates and temporal trends of virus load. J Infect Dis **1999**;180:1018–24.
13. Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1–specific CD4$^+$ T cell responses associated with control of viremia. Science **1997**;278:1447–50.
14. Kirschner DE, Mehr R, Perelson AS. Role of the thymus in pediatric HIV-1 infection. J Acquir Immune Defic Syndr Hum Retrovirol **1998**;18:95–109.
15. Touloumi G, Hatzakis A, Rosenberg PS, O'Brien TR, Goedert JJ. Effects of age at seroconversion and baseline HIV RNA level on the loss of CD4$^+$ cells among persons with hemophilia. Multicenter Hemophilia Cohort Study. AIDS **1998**;12:1691–7.